

1. (original) A method for obtaining nucleic acid from a biological sample and binding the nucleic acid to a solid phase, comprising:

contacting the biological sample with a disrupting buffer, wherein the disrupting buffer comprises:

a protease; and

a cationic surfactant;

substantially neutralizing the cationic surfactant; and

binding the nucleic acid to a solid phase.

2. (original) The method of claim 1, wherein the cationic surfactant does not precipitate when the pH of the buffer is raised above pH 8.

3. (original) The method of claim 1, wherein the substantially neutralizing the cationic surfactant comprises removing the cationic surfactant.

4. (original) The method of claim 1, wherein the substantially neutralizing the cationic surfactant comprises adding a second surfactant that substantially neutralizes the cationic surfactant.

5. (original) The method of claim 4, wherein the cationic surfactant is selected from at least one of the group comprising cetyltrimethylammonium bromide (CTAB),

cetyltrimethylammonium chloride (CTACl), tetradecyltrimethylammonium bromide (TTAB), tetradecyltrimethylammonium chloride (TTACl), dodecyltrimethylammonium bromide (DTAB), dodecyltrimethylammonium chloride (DTACl), dodecylethyldimethylammonium bromide (DEDTAB), decyltrimethylammonium bromide (D<sub>10</sub>TAB), and dodecyltriphenylphosphonium bromide (DTPB).

6. (original) The method of claim 4, further comprising adding a salt.
7. (original) The method of claim 6, wherein the salt is selected from at least one of the group comprising NaBr, NaI, NaSCN, LiCl, LiBr, LiI, GuHCl, and GuSCN.
8. (original) The method of claim 6, wherein the salt is CaCl<sub>2</sub> and is present in a concentration of at least 20 mM.
9. (original) The method of claim 4, wherein the second surfactant is a nonionic surfactant.
10. (original) The method of claim 9, wherein the nonionic surfactant is selected from at least one of the group comprising t-octylphenoxypolyethoxyethanol (Triton X-100), polyoxyethylenesorbitan monolaurate (Tween 21), polyoxyethylenesorbitan monopalmitate (Tween 40), polyoxyethylenesorbitan monostearate (Tween 60), polyoxyethylenesorbitan monooleate (Tween 80), polyoxyethylenesorbitan

monotrioieate (Tween 85), (octylphenoxy)polyethoxyethanol (IGEPAL CA-630), triethyleneglycol monolauryl ether (Brij 30), and sorbitan monolaurate (Span 20).

11. (original) The method of claim 9, wherein the nonionic surfactant is polyoxyethylenesorbitan monolaurate (Tween 20).

12. (original) The method of claim 11, wherein the Tween 20 is present in a concentration of at least 4% w/w.

13. (original) The method of claim 12, wherein the Tween 20 is present in a concentration of 20% w/w.

14. (original) The method of claim 1, wherein the protease is selected from at least one of the group comprising subtilisins, subtilases, and alkaline serine proteases.

15. (original) The method of claim 14, wherein the protease is selected from at least one of the group comprising proteinase K, proteinase, R, proteinase T, subtilisin DY, an alkaline serine protease from *Streptomyces griseus*, an alkaline serine protease from *Bacillus lichenformis*, dispase, subtilisin Calsberg, subtilopeptidase A, and thermolysin.

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16. (original) The method of claim 1, wherein the protease is a thermostable protease.
17. (original) The method of claim 16, wherein the thermostable protease is isolated from an organism selected from at least one of the group comprising *Thermus* Rt41A and *Bacillus thermoproteolyticus* rokko.
18. (original) The method of claim 4, wherein the disrupting buffer further comprises a ribonuclease inhibitor.
19. (original) The method of claim 18, wherein the ribonuclease inhibitor is selected from at least one of the group comprising vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenylic acid), zinc sulfate, bromopyruvate, formamide, copper, and zinc.
20. (original) The method of claim 18, wherein the ribonuclease inhibitor is aurintricarboxylic acid.
21. (original) The method of claim 20, wherein the aurintricarboxylic acid is present in a concentration of 10  $\mu$ M.

22. (original) The method of claim 4, further comprising adding a deoxyribonuclease inhibitor.

23. (original) The method of claim 22, wherein the deoxyribonuclease inhibitor comprises a divalent cation chelator.

24. (original) The method of claim 23, wherein the divalent cation chelator is selected from at least one of the group comprising EDTA, EGTA, and DPTA.

25. (currently amended) A method for obtaining nucleic acid from a biological sample and binding the nucleic acid to a solid phase, comprising:

contacting the biological sample with a disrupting buffer, wherein the disrupting buffer comprises:

a protease; and

a cationic surfactant; ~~and~~

binding the nucleic acid to a solid phase; and

eluting the nucleic acid from the solid phase.

26. (original) The method of claim 25, wherein the cationic surfactant is selected from at least one of the group comprising cetyltrimethylammonium bromide (CTAB),

cetyltrimethylammonium chloride (CTACl), tetradecyltrimethylammonium bromide (TTAB), tetradecyltrimethylammonium chloride (TTACl), dodecyltrimethylammonium bromide (DTAB), dodecyltrimethylammonium chloride (DTACl), dodecylethyldimethylammonium bromide (DEDTAB), decyltrimethylammonium bromide (D<sub>10</sub>TAB), and dodecyltriphenylphosphonium bromide (DTPB).

27. (original) The method of claim 25, further comprising adding a salt.

28. (original) The method of claim 27, wherein the salt is selected from at least one of the group comprising NaBr, NaI, NaSCN, LiCl, LiBr, LiI, GuHCl, and GuSCN.

29. (original) The method of claim 27, wherein the salt is CaCl<sub>2</sub> and is present in a concentration of at least 20 mM.

30. (original) The method of claim 25, wherein the protease is selected from at least one of the group comprising subtilisins, subtilases, and alkaline serine proteases.

31. (original) The method of claim 30, wherein the protease is selected from at least one of the group comprising proteinase K, proteinase, R, proteinase T, subtilisin DY, an alkaline serine protease from *Streptomyces griseus*, an alkaline serine protease from *Bacillus licheniformis*, dispase, subtilisin Calsberg, subtilopeptidase A, and thermolysin.

32. (original) The method of claim 25, wherein the protease is a thermostable protease.

33. (original) The method of claim 32, wherein the thermostable protease is isolated from an organism selected from at least one of the group comprising *Thermus* Rt41A and *Bacillus thermoproteolyticus* rokko.

34. (original) The method of claim 25, wherein the disrupting buffer further comprises a ribonuclease inhibitor.

35. (original) The method of claim 34, wherein the ribonuclease inhibitor is selected from at least one of the group comprising vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenylic acid), zinc sulfate, bromopyruvate, formamide, copper, and zinc.

36. (original) The method of claim 34, wherein the ribonuclease inhibitor is aurintricarboxylic acid.

37. (original) The method of claim 36, wherein the aurintricarboxylic acid is present in a concentration of 10  $\mu$ M.

38. (original) The method of claim 25, further comprising adding a deoxyribonuclease inhibitor.

39. (original) The method of claim 38, wherein the deoxyribonuclease inhibitor comprises a divalent cation chelator.

40. (original) The method of claim 39, wherein the divalent cation chelator is selected from at least one of the group comprising EDTA, EGTA, and DPTA.

41. (original) A kit comprising:

a protease;

a cationic surfactant; and

a second surfactant, wherein the second surfactant substantially neutralizes the cationic surfactant.

42. (original) The kit of claim 41, wherein the cationic surfactant does not precipitate when the pH of the buffer is raised above pH 8.



43. (original) The kit of claim 41, wherein the cationic surfactant is selected from at least one of the group comprising cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTACl), tetradecyltrimethylammonium bromide (TTAB), tetradecyltrimethylammonium chloride (TTACl), dodecyltrimethylammonium bromide (DTAB), dodecyltrimethylammonium chloride (DTACl), dodecylethyldimethylammonium bromide (DEDTAB), decyltrimethylammonium bromide (D<sub>10</sub>TAB), and dodecyltriphenylphosphonium bromide (DTPB).

44. (original) The kit of claim 41, wherein the protease is selected from at least one of the group comprising subtilisins, subtilases, and alkaline serine proteases.

45. (original) The kit of claim 44, wherein the protease is selected from at least one of the group comprising proteinase K, proteinase, R, proteinase T, subtilisin DY, an alkaline serine protease from *Streptomyces griseus*, an alkaline serine protease from *Bacillus licheniformis*, dispase, subtilisin Calsberg, subtilopeptidase A, and thermolysin.

46. (original) The kit of claim 41, wherein the protease is a thermostable protease.

47. (original) The kit of claim 46, wherein the thermostable protease is isolated from an organism selected from at least one of the group comprising *Thermus* Rt41A and *Bacillus thermoproteolyticus rokko*.

48. (original) The kit of claim 41, wherein the kit further comprises a ribonuclease inhibitor.

49. (original) The kit of claim 48, wherein the ribonuclease inhibitor is selected from at least one of the group comprising vanadylate ribonucleoside complexes, phenylglyoxal, p-hydroxyphenylglyoxal, polyamines, spermidine, 9-aminoacridine, iodoacetate, bentonite, poly[2'-O-(2,4-dinitrophenyl)]poly(adenylic acid), zinc sulfate, bromopyruvate, formamide, copper, and zinc.

50. (original) The kit of claim 48, wherein the ribonuclease inhibitor is aurintricarboxylic acid.

51. (original) The kit of claim 50, wherein the aurintricarboxylic acid is present in a concentration of 10  $\mu$ M.

52. (original) The kit of claim 41, wherein the kit further comprises a deoxyribonuclease inhibitor.

53. (original) The kit of claim 52, wherein the deoxyribonuclease inhibitor comprises a divalent cation chelator.

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54. (original) The kit of claim 53, the divalent cation chelator is selected from at least one of the group comprising EDTA, EGTA, and DPTA.
55. (original) The kit of claim 41, further comprising a salt.
56. (original) The kit of claim 55, wherein the salt is selected from the group comprising NaBr, NaI, NaSCN, LiCl, LiBr, LiI, GuHCl, and GuSCN.
57. (original) The kit of claim 55, wherein the salt is  $\text{CaCl}_2$ .
58. (original) The kit of claim 57, wherein the  $\text{CaCl}_2$  is present in a concentration of at least 20 mM.
59. (original) The kit of claim 41, wherein the second surfactant is a nonionic surfactant.
60. (original) The kit of claim 59, wherein the nonionic surfactant is selected from the group comprising t-octylphenoxypolyethoxyethanol (Triton X-100), polyoxyethylenesorbitan monolaurate (Tween 21), polyoxyethylenesorbitan monopalmitate (Tween 40), polyoxyethylenesorbitan monostearate (Tween 60), polyoxyethylenesorbitan monooleate (Tween 80), polyoxyethylenesorbitan

monotrioleate (Tween 85), (octylphenoxy)polyethoxyethanol (IGEPAL CA-630), triethyleneglycol monolauryl ether (Brij 30), and sorbitan monolaurate (Span 20).

61. (original) The kit of claim 59, wherein the nonionic surfactant is polyoxyethylenesorbitan monolaurate (Tween 20).

62. (original) The kit of claim 61, wherein the Tween 20 is present in a concentration of at least 4% w/w.

63. (original) The kit of claim 61, wherein the Tween 20 is present in a concentration of 20% w/w.

64. (original) A kit for obtaining nucleic acid from a biological sample comprising:

- a protease;
- a cationic surfactant;
- a non-ionic surfactant, wherein the non-ionic surfactant permits the binding of nucleic acid to a solid phase in the presence of the protease and cationic surfactant;
- and
- a buffer with a high salt concentration.

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